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DISPERSION-COMPENSATED CALIBRATION FOR GEL PERMEATION CHROMATOGRAPHY: THEORY AND UTILIZATION FOR DIRECT AND UNIVERSAL CALIBRATION

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ABSTRACT

This paper describes the error involved in the utilization of nonmonodisperse polymer standards for the calibration of GPC columns. It also demonstrates that the computer technique developed by McCrackin (1) produces a calibration equation from polydisperse standards that avoids this error. This equation, referred to as dispersion-compensated, can successfully be incorporated into the theory of universal calibration to remove the necessity of assuming infinite instrumental resolution. Furthermore, it is shown that only the calibration equation compensated for number-average molecular weights is a true universal form.

INTRODUCTION

The conventional method of calibrating a gel permeation chromatography (GPC) instrument by plotting the weight-average molecular weights of a series of polymer standards against the modal or maximum peak-height positions of their respective chromatograms results in a calibration equation that does not correctly recalculate the weight-average molecular weights of the original chromatograms used in the calibration. This is a consequence of two sources of error: instrumental spreading which produces an apparent increase in the polydispersities of the true molecular-weight distributions, and the misassignment of a weight-average molecular weight to the modal position of a chromatogram which should correspond to an intermediate value between the weight-average and the number-average molecular weights.

To circumvent this problem, McCrackin (1) has recently developed a computerized calibration technique capable of calculating a calibration equation that is a function of the true modal positions of gaussian monodisperse polymer chromatograms and the appropriate dispersion correction term, specific to the polymer/solvent mixture. This is accomplished by determining the most probable position for a weight-average or a number-average molecular weight for a polydisperse sample on its respective chromatogram and performing a regression analysis with similar values in a series of digitalized, input-data chromatograms of various molecular weights. The equation determined by this technique is dispersion-compensated, because it can be used directly to analyze a chromatogram that has not been corrected for instrumental spreading, to obtain an accurate molecular-weight average of the type used in the calibration.

The purpose of this study is to demonstrate the superiority of McCrackin's technique over the common graphical technique, which utilizes an independently obtained dispersion correction, by documenting the nature of the positional errors inherent in the graphical technique. It is also the purpose of this study to present a valid, dispersionally correct universal calibration derived from the dispersion-compensated equations. The theory of universal calibration based on infinite resolution is invalid because it neglects instrumental spreading and is subject to the propagation of serious positional errors.

#### THEORETICAL DEVELOPMENT

##### Direct Calibration

The utilization of GPC to determine the molecular weight averages of polymer samples requires calibrating an instrument for the expected column-residency time, each molecular-weight and determining the instrumental spreading coefficient (2). Residency time is customarily measured in elution volume units by virtue of a constant elution flow rate. The relationship between molecular weight and elution volume is usually approximated by a linear, semilogarithmic function:

$$\ln \tilde{M}(x) = \ln M(x) = D_1(x) - D_2(x) v, \quad (1)$$

where  $M(x)$  is the molecular weight of a monodisperse polymer of type "x,"  $v$  is the elution volume, and the coefficients  $D_1(x)$  and  $D_2(x)$  are constants characteristic of a particular combination of instrument and polymer/solvent type. Equation (1) is the modal-positional calibration equation for a GPC instrument. It specifies the precise elution volume at which the maximum peak-height of a monodisperse polymer's chromatogram occurs. The GPC chromatogram for this species-polymer will be approximated by a gaussian distribution (3),

$$G(v-\hat{v}) = \exp \left[ -\frac{1}{2}(v-\hat{v})^2/u_2(x) \right] / (2\pi u_2(x))^{1/2},$$

where  $\hat{v}$  is the elution volume at which the modal position occurred, and  $u_2(x)$  is the variance of the gaussian distribution. The parameter  $u_2(x)$  is the spreading coefficient of the instrument and has a magnitude that is also dependent on the instrument and the polymer/solvent mixture (4).

By changing the variable of a gaussian chromatogram from elution volume,  $v$ , to molecular weight via Equation (1), a lognormal molecular weight distribution is obtained (5). But, the modal position of a gaussian distribution corresponds to the median position of a lognormal distribution ( $M(x) = \tilde{M}(x)$ ) (6). Hence, once a series of monodisperse polymers has been eluted to determine Equation (1), the chromatograms of the monodisperse species can be analyzed to determine the apparent molecular-weight averages. Therefore, with

$$H(v) = G(v-\hat{v}),$$

$$\bar{M}_{wa}(x) = \int_0^{\infty} H(v) (D_1(x) - D_2(x) v) dv, \quad (2)$$

$$\text{and} \quad \bar{M}_{na}(x) = 1/\int_0^{\infty} H(v) (-D_1(x) + D_2(x) v) dv, \quad (3)$$

the dispersion parameter  $u_2(x)$  and the monodisperse molecular-weight  $M(x)$  will be related to the apparent values through:

$$\bar{M}_{wa}(x) = M(x) \exp\left(\frac{1}{2} D_2^2(x) u_2(x)\right), \quad (4)$$

$$\text{and} \quad \bar{M}_{na}(x) = M(x) \exp\left(-\frac{1}{2} D_2^2(x) u_2(x)\right). \quad (5)$$

If a new dispersion parameter is defined as

$$P(x) = \exp\left(-\frac{1}{2} D_2^2(x) u_2(x)\right), \quad (6)$$

then from either Equation (4) or Equation (5) the dispersion parameter can be measured directly as

$$P(x) = M(x)/\bar{M}_{wa}(x) = \bar{M}_{na}(x)/M(x).$$

A polydisperse chromatogram is described as the sum of several monodisperse species chromatograms:

$$H(v) = \int_0^{\infty} G(v-\hat{v}) W(\hat{v}) d\hat{v},$$

where  $W(\hat{v})$  is the weight-fraction of each separate species, or the molecular weight distribution. If the shapes of the monodisperse species' chromatograms are fairly gaussian and the calibration equation is linear, then regardless of the actual  $W(\hat{v})$ , the apparent molecular-weight averages, determined for a polydisperse sample through Equation (2) and (3), will also be convertible to the actual molecular-weight values by the same instrumental spreading coefficient of Equation (6) (7). Thus, for polydisperse samples:

$$\bar{M}_w(x) = \bar{M}_{wa}(x) P(x), \quad (7)$$

$$\text{and} \quad \bar{M}_n(x) = \bar{M}_{na}(x) P^{-1}(x). \quad (8)$$

These equations, however, rely on an accurate determination of the modal-positional calibration equation, which predicts the chromatographic position of  $\tilde{M}(x)$ . When real polydisperse calibration standards are utilized, only the weight-average or the number-average or both molecular weights are known, but not the median value  $\tilde{M}(x)$ . Generally, very narrow calibration samples are chosen where  $\bar{M}_w(x) \approx \bar{M}_n(x)$  so that the error between either values and the median values would be very small. But still, an error will be propagated if either  $\bar{M}_w(x)$  or  $\bar{M}_n(x)$  is substituted for  $\tilde{M}(x)$ .

The magnitude of this error can be estimated by considering lognormal  $W(\hat{v})$  functions. For such polymers, ideal infinitely resolved GPC chromatograms, where  $u_2(x) = 0$ , would be true depictions of  $W(\hat{v})$  and would directly yield

$$\bar{M}_w(x) = \tilde{M}(x) \exp\left(\frac{1}{2} D_2^2(x) \sigma^2(x)\right), \quad (9)$$

and 
$$\bar{M}_n(x) = \tilde{M}(x) \exp\left(-\frac{1}{2} D_2^2(x) \sigma^2(x)\right), \quad (10)$$

where  $\sigma^2(x)$  is the actual variance of the distribution. Thus, as a result

$$\bar{M}(x) = (\bar{M}_w(x) \bar{M}_n(x))^{1/2},$$

and 
$$\bar{M}_w(x)/\bar{M}_n(x) = \exp(D_2^2(x) \sigma^2(x)), \quad (11)$$

but, when  $u_2(x) > 0$ , these chromatograms would still be gaussian because of the reproductive properties of gaussian distributions (8,9), although demonstrating empirical variances  $\sigma_v^2(x) > \sigma^2(x)$ . Therefore,

$$\bar{M}_{wa}(x) = \tilde{M}(x) \exp\left(\frac{1}{2} D_2^2(x) \sigma_v^2(x)\right), \quad (12)$$

$$\bar{M}_{na}(x) = \tilde{M}(x) \exp\left(-\frac{1}{2} D_2^2(x) \sigma_v^2(x)\right).$$

The notable feature is that  $\tilde{M}(x)$  would remain constant such that

$$\bar{M}(x) = (\bar{M}_{wa}(x) \bar{M}_{na}(x))^{1/2} = (\bar{M}_w(x) \bar{M}_n(x))^{1/2}.$$

Assigning a true molecular-weight average to a modal position will result in a positional error whose magnitude involves the actual variance of the molecular-weight distributions. By substituting Equation (1) into both Equation (9) and Equation (10), the calibration equations determined from a positional error are:

$$\ln \bar{M}_w^{\rightarrow}(x) = D_1(x) - D_2(x)v + \frac{1}{2} D_2^2(x) \sigma^2(x), \quad (13)$$

and 
$$\ln \bar{M}_n^{\rightarrow}(x) = D_1(x) - D_2(x)v - \frac{1}{2} D_2^2(x) \sigma^2(x), \quad (14)$$

where  $\bar{M}_w^{\rightarrow}(x)$  and  $\bar{M}_n^{\rightarrow}(x)$  are the molecular-weight averages uncorrected for both dispersional and positional errors. Both Equation (13)

and Equation (14) will be referred to as positional-error calibration equations.

A direct conclusion from either Equation (13) or Equation (14) is that narrow, fractionated polymers will minimize the positional error because  $\sigma^2(x) \approx 0$ . Broader calibration standards, whose maximum chromatogram peaks are less likely to be determined precisely, will yield proportionately greater errors as  $\sigma^2(x)$  increases. But,  $\sigma^2(x)$  may also randomly vary within the actual calibration standard series, introducing an error that could possibly exhibit a discernible functionality with elution volume.

To avoid this positional error, McCrackin's computation technique analyzes each calibrating polymer's chromatogram for its shape and determines a calibration equation that will directly recalculate its true weight-average or number-average molecular weight (1). The forms of these new calibration equations are determined by substituting Equation (2) and Equation (3) into Equation (7) and Equation (8), respectively, yielding:

$$\bar{M}_w(x) = \int_0^{\infty} H(v) (D_1(x) - D_2(x) v + \ln P(x)) dv,$$

$$\text{and } \bar{M}_n(x) = 1 / \int_0^{\infty} H(v) (-D_1(x) + D_2(x) v + \ln P(x)) dv.$$

The resulting calibration equations are:

$$\ln \bar{M}_w(x) = D_1(x) - D_2(x) v + \ln P(x), \quad (15)$$

$$\text{and } \ln \bar{M}_n(x) = D_1(x) - D_2(x) v - \ln P(x), \quad (16)$$

which will be referred to as dispersion-compensated calibration (DCC) equations. As is evidenced, DCC equations are the result of a symmetric dispersion operation about the modal-positional calibration equation.

The advantage of McCrackin's calibration technique is that a calibration equation will always be positionally and dispersionally correct regardless of whether the actual calibration-polymer distributions were broad or narrow. Furthermore, it also represents a greatly simplified method procedurally, because no independent dispersion determination is necessary and positional accuracy is

assured even when only one type of molecular-weight average is known, since Equation (15) and Equation (16) are calculated independently of one another.

### Universal Calibration

The technique of universal calibration, in which the calibration equation for one type of polymer is translated into a calibration equation for another type of polymer, is based on the theory that GPC separates according to the solvated hydrodynamic volume of polymers rather than their molecular weights (9). Hydrodynamic volume is defined as the product of the intrinsic viscosity and the molecular weight of a monodisperse polymer,  $[\eta]_x M(x)$ , or simply in terms of molecular weight (through the Mark-Houwink constants  $K_x$  and  $a_x$ ) as  $K_x M^{1+a_x(x)}$ . The prediction for GPC is that two polymers of different molecular weights but identical hydrodynamic volumes will elute at the same elution volume. Ideally then, the modal-positional equation for one polymer should be convertible to the second polymer by manipulation of the Mark-Houwink constants. But, this is theoretically only valid for infinite resolution (9). As demonstrated previously (Equations 4 and 5), the point of elution for a monodisperse polymer is defined as the modal position of the monodisperse species' chromatogram. Therefore, the possibility that two monodisperse polymers with equal mean hydrodynamic volumes, but dissimilar spreading coefficients, may have different median values for their hydrodynamic volume distributions and thus different modal values for their respective chromatograms is not considered. Furthermore, the reality of polydispersity and its potential for producing a positional error is also ignored by this theory.

Significantly, the empirical basis for a universal calibration has been the repeated observation (9-12) that the product of the weight-average molecular weight and the intrinsic viscosity for a series of polydisperse polymers will be colinear in respect to modal elution volumes, with the same product for a second series of polydisperse polymers of differing Mark-Houwink constants,



$$[\eta]_1 \overline{M}_w(1) = [\eta]_2 \overline{M}_w(2). \quad (17)$$

Equation (17), however, has been interpreted as only an approximation to reality where the weight-averages are actually very close in value to the number-averages. But, what is important is that each elution volume increment of a polymer solution after separation will be isolated as a polydisperse solution due to noninfinite resolution. Therefore, each elution volume increment will have an intrinsic viscosity which measures a number-average hydrodynamic volume (13-14) and a viscosity-average molecular weight. Because the viscosity-average molecular weight is frequently approximated by the weight-average molecular weight, Equation (17) actually predicts that the weight-average of each elution volume increment for one polymer type will be proportional to the weight-average for a second polymer type.

$$K_1 \overline{M}_w^{-1+a_1}(1) = K_2 \overline{M}_w^{-1+a_2}(2). \quad (18)$$

In effect, a weight-average DCC equation describes the weight-average molecular weight that each elution volume increment will have for a polymer solution that has been separated with a particular dispersion coefficient of  $P(x)$ . This is the reason that a GPC chromatogram can be analyzed directly with such an equation to yield an accurate weight-average for the entire polymer or polymer solution. Thus, Equation (18) at first inspection seems capable of interconverting one weight-average DCC equation directly into another. But, the specific dispersion coefficient that one polymer type may have is independent of the other. Therefore, although dispersion coefficients are not translatable, the equality of Equation (18) still remains, allowing the modal-positional equation of polymer "1" to be described in terms of the modal-positional equation of polymer "2" and both dispersion coefficients. Thus, by substituting Equation (15) for both polymers into Equation (18):

$$\ln \overline{M}(1) = \alpha + \beta \ln \overline{M}(2) - \beta \ln P(2) + \ln P(1), \quad (19)$$

where

$$\alpha = \ln (K_2/K_1) / (1 + a_1),$$

$$\text{and} \quad \beta = (1 + a_2) / (1 + a_1).$$

What is notable about Equation (19) is that when  $\ln P(1) = \ln P(2) = 0$ , the standard calibration equation transformation utilized for universal calibration, known as the Coll-Prusinowski formalism (15,16), is obtained. Thus, when a true modal-positional calibration equation for polymer "2" is translated via the Coll-Prusinowski formalism, both dispersion coefficients of Equation (19) are neglected. This neglect would be inconsequential if instrumental resolution of both polymers was infinite, or if  $\beta \ln P(2) = \ln P(1)$ . But, since the former condition is impossible and the latter, at best, serendipitous, at least a dispersional error will always be expected.

Analogously, the application of the Coll-Prusinowski formalism to a positional-error equation [Equation (13)], derived from the assignment of weight-average molecular weights to the modal positions of the calibration chromatograms, results in included errors that also rely on fortuitous relationships between the positional error and the dispersional error for minimization.

Thus, to avoid both dispersional errors and positional errors, it would be advantageous to be able to translate a DCC equation for polymer "2" directly into a DCC equation for polymer "1". Applying the principle of dispersion compensation to the modal-positional Equation (19) yields:

$$\ln \tilde{M}_w(1) = \alpha + \beta \ln \tilde{M}(2) - \beta \ln P(2) + 2 \ln P(1), \quad (20)$$

$$\text{and} \quad \ln \bar{M}_n(1) = \alpha + \beta \ln \tilde{M}(2) - \beta \ln P(2). \quad (21)$$

The elimination of the dispersion coefficient  $P(1)$  in Equation (21) is noteworthy because this parameter could not be derived without independent dispersion measurements. When both DCC equations for polymer "2" (Equations 15 and 16) are translated directly by the Coll-Prusinowski formalism, the results are:

$$\ln \bar{M}_w(1) = \alpha + \beta \ln \tilde{M}(2) + \beta \ln P(2), \quad (22)$$

$$\text{and} \quad \ln \bar{M}_n(1) = \alpha + \beta \ln \tilde{M}(2) - \beta \ln P(2). \quad (23)$$

The comparison of Equation (21) and Equation (23) demonstrates that the number-average DCC equation is a true universal form and can be translated into the number-average DCC equation for any other polymer whose Mark-Houwink constants are known, through the Coll-Prusinowski formalism. The comparison of Equation (20) and Equation (22) demonstrates that the weight-average DCC equation will not translate through the Coll-Prusinowski formalism. When one such weight-average equation is translated, a dispersion error will result between what is obtained and the true relationship.

#### METHODS

Waters-brand Styragel columns with the nominal exclusion limits of  $10^2$ ,  $10^3$ ,  $10^4$ ,  $10^5$ , and  $10^6 \text{ \AA}$  were utilized with purified tetrahydrofuran (THF) as the elution solvent. The flow rate was 2 mL/min. A Perkin-Elmer LC-55B spectrophotometer was operated at 235 nm for the cellulose tricarbonylates and 225 nm for the polystyrene calibration standards. The polystyrene standards were obtained from the manufacturers listed in Table I. Cellulose tricarbonylates were prepared and injected into the GPC columns according to the methods of Schroeder and Haigh (17).

All calculations were performed by computer programs obtained from McCrackin. Two programs are available: Program Calib calibrates GPC columns by the method described in (1), and program GPC computes the molecular weight averages from chromatograms of polymer samples.

#### RESULTS AND DISCUSSION

##### Direct Calibration

The chromatograms of ten polystyrene samples which were utilized as calibration standards are depicted in Fig. 1. Each chromatogram has been normalized to the same area and has been characterized by the calculation of its statistical distribution parameters: in particular, its mean ( $\bar{V}$ ), median ( $\tilde{V}$ ), mode ( $\hat{V}$ ), and the standard deviation ( $\sigma_V$ ) with respect to elution volume. These values for each chromatogram are listed in Table 1 along with

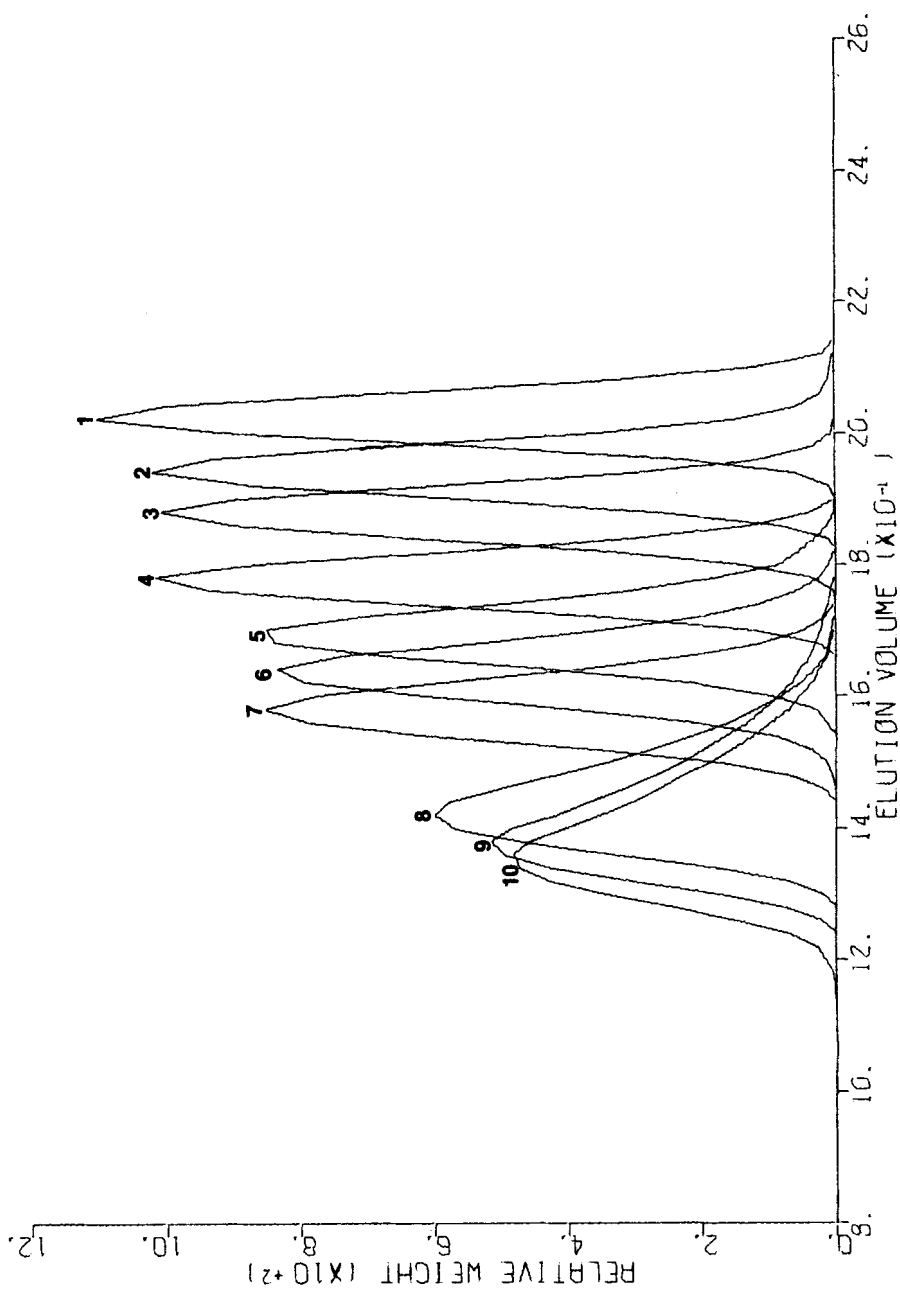


Figure 1. The GPC chromatograms of ten polystyrene calibration standards.

TABLE 1  
Vendor-Supplied Weight-Average Molecular Weights  
and GPC Statistical Chromatographic Parameters

Sample	Supplement	$\overline{M}_w$ [vs]	$\overline{V}$	$\tilde{V}$	$\hat{V}$	$\sigma_v$	sk
1	PCC	$4.80 \times 10^3$	202	202	202	3.49	0.0
2	PCC	$1.03 \times 10^3$	194	194	194	3.88	0.0
3	PCC	$1.98 \times 10^4$	188	188	188	3.85	0.0
4	PCC	$5.10 \times 10^4$	178	178	178	3.81	0.0
5	DPR	$1.25 \times 10^5$	170	170	170	4.85	0.0
6	PIB	$2.39 \times 10^5$	164	164	164	4.95	0.0
7	WA	$4.70 \times 10^5$	158	158	158	4.62	0.0
8	PCC	$1.80 \times 10^6$	145	144	142	6.98	0.430
9	WA	$2.70 \times 10^6$	143	141	138	9.13	0.548
10	PIB	$3.56 \times 10^6$	139	138	136	8.82	0.340

PCC = Pressure Chemical Company.

DPR = Dow Physical Research Lab.

PIB = Polytechnic Institute of Brooklyn.

WA = Waters Associates.

their respective vendor-supplied, weight-average molecular weights,  $\overline{M}_w$ [vs].

The modal value  $\hat{V}$ , is the parameter customarily selected for graphical calibration of GPC instruments because it corresponds to the easily identifiable maximum peak-height of a chromatogram. Both  $\overline{V}$  and  $\tilde{V}$  will be equal when the chromatograms are symmetrical, but all three parameters will not be equal when skewing is present. The degree of skewing for each chromatogram is measured by

$$sk = (\overline{V} - \tilde{V}) / \sigma_v,$$

and, as can be seen from Table 1, chromatograms No. 1 through 7 are symmetric, since  $\overline{V} = \tilde{V} = \hat{V}$  and  $sk = 0$ ; however, for chromatograms No. 8, 9, and 10,  $\overline{V} > \tilde{V} > \hat{V}$  and  $sk > 0$ . They are obviously nongaussian. Specifically, this means that for the skewed chromatograms, the modal elution volume will not correspond positionally to  $\tilde{M}(x)$ . Thus, a modified positional-error equation will result

from their inclusion in a graphical calibration. The extent of this modification can be estimated by assuming that  $\tilde{M}(x)$  may possibly reside closer to either  $\bar{V}$  or  $\tilde{V}$ , and that the positional-error equation determined from the symmetric chromatograms is valid through the skewed chromatograms.

For all ten polystyrene chromatograms, three calibration equations were derived from a linear regression of the logarithm of the vendor-supplied weight-average molecular weight and the elution volume parameters. The three calibration equations obtained were:

$$\begin{aligned} \ln \bar{M}_w[\bar{V}] &= 29.87 - 0.1064 \bar{V}, \\ \ln \bar{M}_w[\tilde{V}] &= 29.42 - 0.1040 \tilde{V}, \\ \text{and} \quad \ln \bar{M}_w[\hat{V}] &= 28.67 - 0.09987 \hat{V}, \end{aligned} \quad (24)$$

but, the single calibration equation that was determined from the symmetric chromatograms No. 1 through 7 was

$$\ln \bar{M}_w[\tilde{V}] = 29.45 - 0.1041 \tilde{V}.$$

Since Equation (25) is approximately equal to Equation (24), the median elution volume  $\tilde{V}$  is assumed to be positionally correct for  $\bar{M}(x)$ . Thus, the observable effect of utilizing either  $\bar{V}$  or  $\tilde{V}$  for the skewed chromatograms instead of  $\tilde{V}$  is that linear error functions are included that affect both the slope and intercept of Equation (24).

The dispersion-compensated calibration equation obtained via McCrackin's computer program for all ten chromatograms was

$$\ln \bar{M}_w[t] = 28.93 - 0.1017 v. \quad (26)$$

It varies in both slope and intercept from Equation (24). If Equation (24) were a true modal-positional calibration equation, the expectation is that only the intercepts would differ due to the inclusion of the constant dispersional parameter  $\ln P(x)$  in Equation (26); however, because of the positional error of assigning the weight-average molecular weight to a modal position, the actual form of Equation (24) is given by Equation (13). But,

by substituting Equations (6), (9), and (12) into Equation (7) and simplifying, the standard relationship between dispersion and distribution variances for the symmetrical chromatograms was obtained (18):

$$\sigma^2(x) = \sigma_v^2(x) - u_2(x). \quad (27)$$

By further substitution of Equation (27) into Equation (13), the positional-error equation can be compared with the DCC equation,

$$\ln \overline{M}_w'(x) = \ln \overline{M}_w(x) + \frac{1}{2} D_2^2(x) \overline{\sigma}_v^2. \quad (28)$$

Thus, the fact that the parameter  $\sigma_v$  actually does show a functionality with elution volume in Table 1 explains why the slope of Equation (24) was also different from the slope of Equation (26). But, more importantly, Equation (28) demonstrates that any weight-average molecular weight calculated from a positional-error equation will always be greater than the weight-average calculated from a true modal-positional equation with dispersion correction performed either independently or simultaneously, as in McCrackin's method. This is true because no distribution variance  $\sigma^2(x)$  can be obtained for any polymer standards that will yield an empirical  $\overline{\sigma}_v^2$  equal to zero. Therefore,  $\overline{M}_w'(x)$  is a weight-average molecular weight that is uncorrected for dispersion and positional errors, and will still yield incorrect values when corrected for just peak dispersion.

Table 2 compares the vendor-supplied weight-average molecular weights with the weight-averages calculated for each polystyrene chromatogram by all four calibration equations mentioned, in a numerical approximation of Equation (2). As expected, the dispersion-compensated calibration equation (Equation 26) demonstrated calculated weight-average values  $\overline{M}_w[t]$  with an insignificant average deviation from the vendor-supplied values. In contrast, all three graphical methods of calibration produced larger average deviations. The values calculated from the median calibration (Equation 24)  $\overline{M}_w[\tilde{V}]$  were consistently larger than the values calculated from McCrackin's calibration  $\overline{M}_w[t]$ , as predicted

TABLE 2  
Calculated Weight Averages

Sample	$\bar{M}_w$ [vs]	$\bar{M}_w$ [t]	$\Delta\%a$	$\bar{M}_w$ [V]	$\Delta\%a$	$\bar{M}_w$ [ $\hat{V}$ ]	$\Delta\%a$	$\bar{M}_w$ [ $\hat{V}$ ]	$\Delta\%a$
1	$4.80 \times 10^3$	$4.54 \times 10^3$	-5.4	$4.55 \times 10^3$	-5.2	$4.67 \times 10^3$	-2.7	$5.04 \times 10^3$	5.0
2	$1.03 \times 10^4$	$1.02 \times 10^4$	-1.0	$1.04 \times 10^4$	2.9	$1.07 \times 10^4$	3.9	$1.12 \times 10^4$	8.7
3	$1.98 \times 10^4$	$2.00 \times 10^4$	1.0	$2.15 \times 10^4$	8.6	$2.13 \times 10^4$	7.6	$2.16 \times 10^4$	9.1
4	$5.10 \times 10^4$	$5.51 \times 10^4$	8.0	$6.20 \times 10^4$	21.6	$6.00 \times 10^4$	17.6	$5.85 \times 10^4$	14.7
5	$1.25 \times 10^5$	$1.32 \times 10^5$	5.6	$1.55 \times 10^5$	24.0	$1.47 \times 10^5$	17.6	$1.38 \times 10^5$	10.4
6	$2.39 \times 10^5$	$2.38 \times 10^5$	-0.4	$2.88 \times 10^5$	20.5	$2.69 \times 10^5$	12.6	$2.46 \times 10^5$	2.9
7	$4.70 \times 10^5$	$4.18 \times 10^5$	-11.1	$5.17 \times 10^5$	10.0	$4.77 \times 10^5$	1.5	$4.28 \times 10^5$	-8.9
8	$1.80 \times 10^6$	$1.84 \times 10^6$	2.2	$2.45 \times 10^6$	36.1	$2.18 \times 10^6$	21.1	$1.83 \times 10^6$	1.7
9	$2.70 \times 10^6$	$2.55 \times 10^6$	-5.6	$3.46 \times 10^6$	28.1	$3.05 \times 10^6$	13.0	$2.52 \times 10^6$	-6.7
10	$3.56 \times 10^6$	$3.65 \times 10^6$	2.5	$5.04 \times 10^6$	41.6	$4.40 \times 10^6$	23.6	$3.59 \times 10^6$	0.8
			$\overline{\Delta\%}$		18.8		11.6		3.8
			$\sqrt{\overline{\Delta\%}}$		4.3		12.1		6.9

$\overline{\Delta\%}$  Relative error based on  $\bar{M}_w$  [vs] values.



by Equation (28). The effect of positional discrepancy between the modal elution volume  $\hat{V}$  and the value of  $\tilde{M}(x)$  from the skewed chromatograms is depicted by the  $\overline{M}_w[\hat{V}]$  values for chromatograms No. 8, 9, and 10. When skewing is positive ( $sk > 0$ ), the modal value reduces the effect of positional error and produces values very close to both the dispersion-compensated and the vendor-supplied.

The substitution of Equation (27) into Equation (11) results in an expression that may be used to calculate the number-average molecular weight of each calibrating polymer's chromatogram,

$$\overline{M}_n(x) = \overline{M}_w(x) \exp(-D_2^2(x) \sigma_v^2 / P^2(x)).$$

Knowledge of the spreading coefficient  $P(x)$ , however, is required. But, McCrackin's technique can also be used in an iterative fashion to determine which value of  $P(x)$  will yield a number-average DCC equation that is parallel to the previously determined weight-average DCC equation. For various values of  $P(x)$ , Table 3 demonstrates the response of the computer program in calculating the new number-average DCC equations. Since McCrackin's technique is not restricted to linear equations, quadratic coefficients will occur when included-error functions are also nonlinear. As can be seen in Table 3, the best value of  $P(x)$  which gave the lowest

TABLE 3  
Number-Average DCC Equations

P	$D_1(2)$	$D_2(2)$	$D_3(2)$	RE <sup>a</sup>
0.99	29.00	-0.1022	$1.185 \times 10^{-6}$	0.61
0.98	29.02	-0.1021	$1.154 \times 10^{-6}$	0.47
0.97	29.01	-0.1018	0.0	0.42
0.96	29.03	-0.1018	$4.240 \times 10^{-9}$	0.59
0.95	29.09	-0.1023	$1.458 \times 10^{-6}$	0.46

<sup>a</sup>Relative error determined by McCrackin's computer program by comparing number-average values against inputted number-average values.

relative error, and a number-average DCC equation approximately parallel to the weight-average DCC equation was  $P(x) = 0.97$ .

The substitution of Equation (27) into Equation (11) results in an expression that may be used to calculate the number-average molecular weight of each calibrating-polymer's chromatogram,

$$\bar{M}_n(x) = \bar{M}_w(x) \exp(-D_2^2(x) \sigma_v^2) / P^2(x).$$

Knowledge of the spreading coefficient  $P(x)$ , however, is required. But, McCrackin's technique can also be used in an iterative fashion to determine which value of  $P(x)$  will yield a number-average DCC equation that is parallel to the previously determined weight-average DCC equation. For various values of  $P(x)$ , Table 3 demonstrates the response of the computer program in calculating the new number-average DCC equations. Since McCrackin's technique is not restricted to linear equations, quadratic coefficients will occur when included-error functions are also nonlinear. As can be seen in Table 3, the best value of  $P(x)$  which gave the lowest relative error, and a number-average DCC equation approximately parallel to the weight-average DCC equation was  $P(x) = 0.97$ .

In this calculation,  $\bar{M}_w[t]$  values should be used for  $\bar{M}_w(x)$  instead of  $\bar{M}_w[vs]$  because  $\bar{M}_n[t]$  values are needed to complement the respective weight-averages. Therefore, the experimental errors associated with  $\bar{M}_w[vs]$  values do not enter into these calculations.

Utilizing Equation (25) to analyze the chromatogram of polystyrene SRM-706 (19),  $\bar{M}_w[t] = 2.80 \times 10^5$  was calculated. This value fell between the weight-average values reported for light scattering ( $2.58 \times 10^5$ ) and sedimentation equilibrium ( $2.88 \times 10^5$ ).

The number-average molecular weight calculated at  $P(x) = 0.97$  gave  $\bar{M}_n[t] = 1.49 \times 10^5$ , which may be compared with the membrane osmometry number-average of  $1.37 \times 10^5$ . The calculated GPC polydispersity ( $\bar{M}_w[t] / \bar{M}_n[t] = 1.88$ ) was identical to the polydispersity calculated from the ratio of the light scattering to the membrane osmometry values; however, both the calculated weight-average and number-average molecular weights have a relative error

of 8% greater than the direct measurement. This error is within acceptable limits for GPC.

### Universal Calibration

With the intention of obtaining DCC equations for cellulose tri-carbanilates (CTC), both the weight-average and the number-average DCC equations for polystyrene were translated via the Coll-Prusinski formalism. The constants  $\alpha$  and  $\beta$  were calculated using the respective Mark-Houwink constants [ $K_2 = 1.179 \times 10^{-2}$  and  $a_2 = 0.74$  for polystyrene,  $K_1 = 2.010 \times 10^{-3}$  and  $a_1 = 0.92$  for CTC (10)]. In this situation, however, it was found necessary to subtract the Ptitsyn-Eisner correction factor (0.15) from  $\alpha$  to account for CTC's deviations from random coiling (14,20). The discrepancy that was discovered involved the calculation of a number-average greater than the vendor-supplied weight-average for the narrowest sample (N-5) when the correction was not included.

The resulting calibration equations were:

$$\ln \bar{M}_w(1) = 26.99 - 0.09217 v, \quad (29)$$

and 
$$\ln \bar{M}_n(1) = 27.06 - 0.09226 v. \quad (30)$$

As expected and demonstrated in Table 4, the weight-average values  $\bar{M}_w[T]$  calculated for the CTC chromatograms via Equation (29) differed from the vendor-supplied weight-average values  $\bar{M}_w[vs]$ . By virtue of the difference between Equation (20) and Equation (22), the dispersional error would be:

$$P^2(1) / P^{2\beta}(2) = \bar{M}_w[vs] / \bar{M}_w[T].$$

From Table 4 and with  $P(2) = 0.97$ , the dispersional coefficient was found to have an average value of  $P(1) = 0.67$ . This smaller  $P(x)$  value for CTC is consistent with a greater degree of dispersion due to a greater rigidity of the polymers in solution (20). The correct DCC equation for the weight-average values was determined to be:

$$\ln \bar{M}_w(1) = 26.26 - 0.09217 v, \quad (31)$$

which yielded much more reasonable values for  $\bar{M}_w[t] \approx \bar{M}_w[vs]$ . The dispersion coefficient calculated from Equation (30) and Equation (31) was  $P(1) = 0.67 = \exp\left(\frac{1}{2} (26.26 - 27.06)\right)$ .

Table 4  
Cellulose Tricarbanilate Data

Sample <sup>a</sup>	$\overline{DP}_w[vs]^b$	$\overline{DP}_w[T]$	P(1) <sup>c</sup>	$\overline{DP}_w[t]$	$\overline{DP}_n[t]$
N-5	550	1130	0.68	542	473
N-30	1000	2310	0.64	1110	816
N-70	1300	2480	0.70	1270	764
		Average	0.67		

<sup>a</sup>Samples were obtained from Hercules, Inc.

<sup>b</sup> $\overline{DP} = M / 519$ .

<sup>c</sup>Calculated from  $P^2(1) = P^2\beta(2) \overline{DP}_w[vs] / \overline{DP}_w[T]$ .

By simple inspection of Equation (24) in regard to Equation (26), the application of the Coll-Prusinowski formalism to the positional-error equation for the polystyrenes would have resulted in weight-average molecular weights even larger than the  $\overline{M}_w[T]$  values. This is a consequence of the incorporation of both dispersional errors and positional errors. This fact can be demonstrated by simple application of the Coll-Prusinowski formalism to Equation (28).

$$\ln \overline{M}_w(1) = \alpha + \beta \ln \tilde{M}(2) + \beta \ln P(2) + \frac{1}{2} \beta D_2^2(2) \sigma_v^2,$$

which is Equation (22) with a positional-error term that will always be greater than zero because  $\sigma_v^2$  will never be zero.

#### SUMMARY

The calculation of reliably accurate molecular-weight averages for polymer samples through gel permeation chromatography depends on the determination of a true modal-positional calibration and an instrumental spreading coefficient. The common graphical procedure of assigning a weight-average molecular weight value to a modal position on a chromatogram will result, however, in an equation with a positional error that will produce calculated weight-averages that are always greater in value than the dispersionally correct value. Because the dispersion correction will generally not be appropriate for the positional error, further correction of these values for dispersional error will not properly correct these

values. To avoid this situation, McCrackin has developed a computerized calibration technique which will calculate a dispersion-compensated calibration equation for either weight-average or number-average molecular weight that is dispersionally and positionally correct. Dispersion-compensated calibration equations can be used to directly analyze a GPC chromatogram to obtain an accurate value for the particular molecular weight-average that was originally used to obtain the equation without further dispersion correction.

The problems of positional and dispersive errors are generally magnified when a calibration equation for one polymer type is converted, by the principle of universal calibration, into the equation for another polymer type whose identical hydrodynamic volume corresponds to a different molecular weight. Because a number-average dispersion-compensated calibration equation is properly balanced for the correct dispersion terms, it is a true universal form and can be translated into the number-average dispersion-compensated equation for another polymer type through a universal calibration transformation.

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CALIBRATION NOMENCLATURE

$a_x$ or $k_x$	The Mark-Houwink exponent or coefficient for polymer "x" determined from the double logarithmic plot of intrinsic viscosity and molecular weight.
$D_j(x)$	The coefficients of a generalized GPC polynomial calibration equation, $j = 1, 2, 3$ , etc.
$G(v-\hat{v})$	The gaussian-shaped GPC chromatogram of a monodisperse polymer.
$H(v)$	The GPC chromatogram for any monodisperse or polydisperse polymer.
$M(x)$	The molecular weight for a monodisperse polymer of type "x."
$\sim$	
$M(x)$	The median value of a molecular weight distribution for a polymer of type "x."
$\bar{M}_w(x)$ or $\bar{M}_n(x)$	The weight- or number-average molecular weight for a polydisperse polymer of type "x."
$\bar{M}_w'(x)$ or $\bar{M}_n'(x)$	The weight- or number-average molecular weight determined by GPC having both positional and dispersional errors.
$\bar{M}_{wa}(x)$ or $\bar{M}_{na}(x)$	The weight- or number-average molecular weight determined by GPC having only a dispersional error.
$\bar{M}_w[t]$ or $\bar{M}_n[t]$	The weight- or number-average molecular weight determined from a GPC chromatogram utilizing a dispersion-compensated calibration equation.
$\bar{M}_w[T]$	The weight-average molecular weight determined from a universal calibration equation with compound positional and dispersional errors for two different types of polymers.

$\bar{M}_w[\bar{V}]$	The weight-average molecular weight determined for a GPC chromatogram with a calibration equation derived from the plot of the weight-average molecular weight <u>versus</u> the mean elution volume of a series of calibration standards.
$\bar{M}_w[\tilde{V}]$	The same as above but from a plot of $\bar{M}_w$ [vs] <u>versus</u> the median elution volume.
$\bar{M}_w[\hat{V}]$	The same as above but from a plot of $\bar{M}_w$ [vs] <u>versus</u> the modal elution volume.
$\bar{M}_w$ [vs]	The vendor-supplied weight-average molecular weight.
$P(x)$	The dispersion correction factor for a polymer of type "x."
sk	The skewing parameter for a GPC chromatogram.
$u_2(x)$	The standard deviation of the chromatogram for a monodisperse polymer.
v	The empirically measured elution volume on a GPC chromatogram.
$\hat{v}$	The elution volume corresponding to a modal position of a monodisperse polymer chromatogram.
$\bar{V}$	The mean elution volume of a GPC chromatogram.
$\tilde{V}$	The median elution volume of a GPC chromatogram.
$\hat{V}$	The modal elution volume of a GPC chromatogram.
$W(\tilde{v})$	A polymer's molecular weight distribution, with the molecular weight variable transformed to the median elution volume by a median-positional calibration equation.
x	A symbol denoting the type of polymer, e.g., the type of monomer it is composed of.
$\alpha$	The intercept of the Coll-Prusinowski formalism.
$\beta$	The slope of the Coll-Prusinowski formalism.
$\sigma(x)$	The standard deviation of the molecular weight distribution of a polymer of type "x."



$\sigma_v$	The standard deviation of a GPC chromatogram, determined from $(\int H(v) (v - \bar{v})^2 / \int H(v))^{1/2}$ .
mean	Of GPC chromatogram: $(\int H(v) v / \int H(v))$ . Of a lognormal molecular-weight distribution: $\ln \bar{M}(x) + \frac{1}{2} D_2^2(x) \sigma^2(x)$ .
median	Of GPC chromatogram: The value of the elution volume, $v$ , corresponding to the vertical line which divides a chromatogram into two parts having equal areas. Of a lognormal molecular-weight distribution: The value that divides the distribution into equal areas, symbolized as $\ln \tilde{M}(x)$ .
mode	Of GPC Chromatogram: The value of the elution volume at the maximum peak height. Of a lognormal molecular-weight distribution: $\ln \tilde{M}(x) - D_2^2(x) \sigma^2(x)$ .